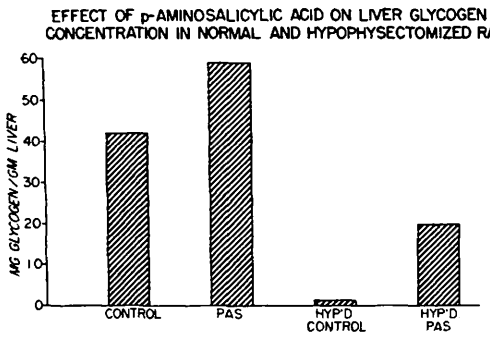

FIG. 4.

fore the radioiodine tracer dose was given.

Our findings substantiate the observation of Cronheim *et al.* that PAS has no effect on the pituitary-adrenal axis, at least when administered over a 2-week period. Although elevation of the liver glycogen concentration was observed in all PAS-treated animals, this is not necessarily an adrenal-mediated response and may be the result of a direct action of PAS in the liver; this is especially interesting in view of the report by Zahn(3) that many patients receiving PAS excrete a reducing substance in their urine. Further work on this phase of the problem is contemplated.

Summary. 1. Administration of PAS to rats over a 2-week period produced goiter associated with diminished I^{131} uptake by the thyroid gland. 2. No effect on the pituitary-adrenal axis was observed. 3. Elevation of liver glycogen concentration was noted in all PAS-treated animals.

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Received March 9, 1953. P.S.E.B.M., 1953, v82.

A Simple Direct Method for Absolute Basophil Leucocyte Count.* (20190)

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Little is known concerning the physiologic and pathologic significance of the basophil. Previous attempts to study these cells have been hindered by the scarcity of basophils in the bone marrow and circulating blood. Slight differences in absolute basophil counts are not easily detectable by indirect methods and at least 1000 leucocytes should be counted to insure a fair sampling of the basophils present. Such counts are also apt to have a large margin of error due to distribution of leucocytes in the stained blood smear. With a direct counting method, one in which the cells are tallied and differentiated in the same

* Presented at Am. Fed. for Clinical Research, 1952, New York City.

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counting chamber, these difficulties would not be encountered. Such a method is described in this paper.

The method employs the metachromatic staining principle described by Ehrlich(1), in which the metachromatic material takes a different color than might be expected of the stain. The granules in the cytoplasm of the basophil contain a metachromatic substance. Of the various metachromatic stains tested, toluidine blue appeared to be the most suitable and stained the basophil granules a bright pink to reddish-violet color. The nuclei of all leucocytes, including the basophil, stained bluish-violet differing only in intensity of staining as to the type of cell. The eosinophils can easily be distinguished by their larger size, the greenish-yellow appearance of their cytoplasm and granules, and by the rounded bi-lobed nuclei. The significant point is that only the basophils take a metachromatic stain.

Method. A diluent was needed which would destroy or hemolyze the erythrocytes and one which would not dissolve the water-soluble basophilic granules. Inorganic acids and bases, acetic acid, propylene glycol, and several short-chained alcohols were tested as diluents. Acetic acid and the inorganic acids were effective hemolyzing agents, but the basophilic granules were soluble in these reagents. Inorganic bases hemolyzed not only the erythrocytes, but destroyed the leucocytes as well. Propylene glycol was a good hemolyzing agent, but it failed to fix the basophilic granules(2). The short-chained alcohols in concentrations of 15-30% were effective in fixing the granules, but they were ineffective as hemolyzing agents. A solution of saponin in 20% ethanol was found to best meet the requirements of the diluent needed for the proper staining of these cells. Table I gives the composition of the fluid used in this study. Fresh unclotted blood obtained by finger puncture or citrated, or oxalated, venous blood is diluted with the above counting fluid in a proportion of one to 10 in the standard leucocyte pipette. The pipette is shaken either mechanically or manually for 3 minutes. The 4 chambers of 2 Fuchs-Rosenthal (0.2 mm deep) hemocytometers are filled by capillary action in the usual manner. The counting chambers are allowed to stand a few minutes so that the leucocytes may settle. They should be placed into a large covered Petri dish into which a dampened piece of blotting paper has been placed to insure against excessive drying, and while one hemocytometer is being counted, the other is left within the moistened atmosphere. The high magnification (4 mm objective) is used and all 4 chambers are counted. Not enough contrast between the metachromatic staining granules and the nuclei of the other cells is obtained to permit differentiation of the basophil under a low magnification (16 mm objective). The number of basophils counted in all 4 chambers is totaled, averaged, and multiplied by the dilution factor, 3.13, which gives the absolute count in terms of basophils per cubic millimeter. A total leucocyte count per cu mm may be done simultaneously by counting 2 of the 16 larger squares, averaging, and multiplying by 50. It has been found best to fill the chambers immediately after shaking for if the pipette stands longer than 30 minutes, a precipitate may appear which forms around the leucocytes making accurate counting impossible.

TABLE I.
Composition of Staining and Diluting Fluid.

	ml
0.05% toluidine blue in 0.85% saline	40
95.0% ethyl alcohol	11
Saturated sol. of saponin in 50% ethyl alcohol	1

Mix well and filter before use. This solution will keep at least 4 months at room temp. Occasional filtering may be necessary to keep the solution free from sediment.

Results. Satisfied that this method was reproducible, correlation was made between the direct (absolute) basophil counts and indirect basophil counts (calculated from the total leucocyte count, and from the percentage of basophils on the peripheral blood smear after counting 4000 leucocytes). The total white count was taken from the same chamber in which the basophil count was determined. This procedure was carried out on 31 individuals; 11 normal males, 9 normal females, and 11 individuals with miscellaneous diseases. Fig. 1 demonstrates this correlation.

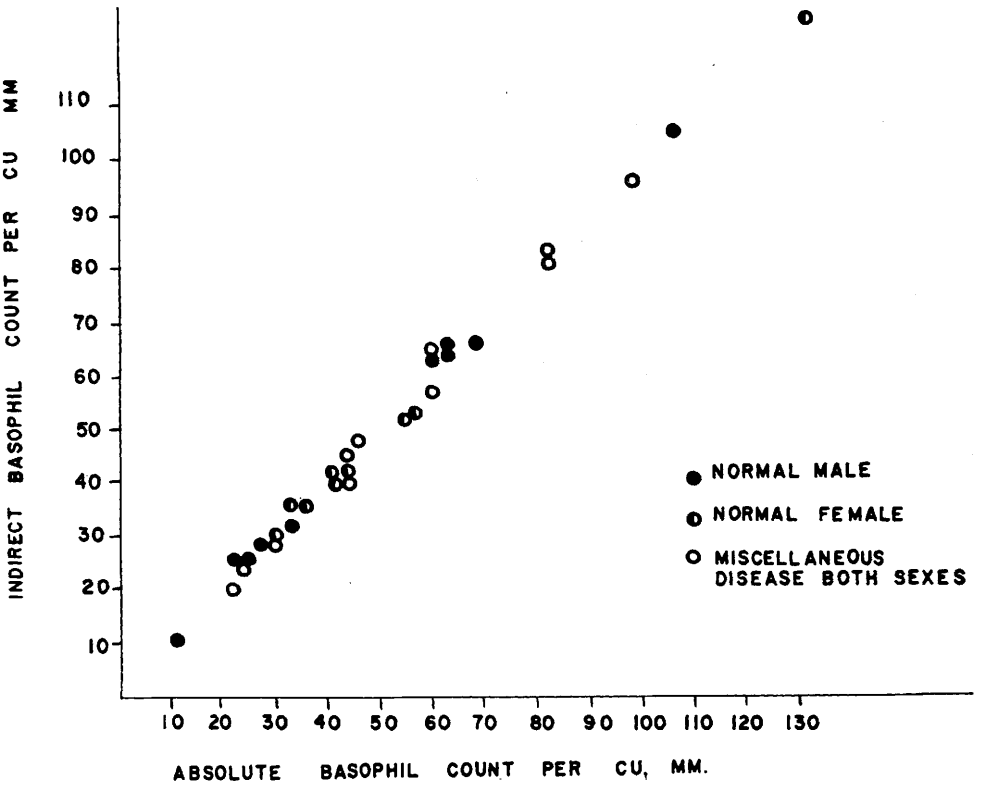

FIG. 1.

There appears to be an excellent agreement.

Normal values were determined on each of 36 young males and 33 females, all apparently healthy individuals who were either house officers, medical students, nurses, or technical assistants. In the male group, ranging in age from 20 to 38 years, the mean absolute basophil count was 46.7 ± 20.1 (S.D.) per cubic millimeter. The counts ranged from 11 to 107. In the female group, ranging in age from 18 years to 34 years, the mean absolute basophil count was 40.6 ± 18.9 (S.D.) per cubic millimeter. The total count ranged from 8 to 88 cells per cubic millimeter. There is no statistical difference between the means in these 2 groups. These data are summarized in Table II.

Discussion. This method now affords a means of studying changes in the absolute basophil counts in a variety of clinical disorders. Heretofore, the time-consuming necessity of counting at least 1000-2000 white cells has inhibited much needed investigation of the function and fluctuation in the basophil leucocyte. Changes in total basophils can be correlated with other physiological studies in the same manner as changes in the absolute eosinophils have been investigated. Studies of this nature are now in progress in this laboratory.

TABLE II. Normal Values for Basophils/mm³.

Sex	No.	Age	Mean	S.D.	Range
♂	36	20-38	46.7	±20.1	11-107
♀	33	18-34	40.6	±18.9	8-88
No significant sex difference t = .26					

Summary. A method is presented which permits the differentiation and enumeration of basophils from the counting chamber. Basophil counts were performed on 69 normal individuals and the range, mean, and standard deviation are reported by sex.

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Received March 10, 1953. P.S.E.B.M., 1953, v82.